IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	Group Art Unit: 1648
COX	Examiner: Lucas, Z.
Serial No.: 10/773,530	Confirmation No.: 7322
Filed: February 5, 2004	DECLARATION OF GEORGE COX
Atty. File No.: 4152-1-PUS-9	
For: "CYSTEINE VARIANTS OF ERYTHROPOIETIN"	**Submitted Electronically**

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Dear Sir:

I, George Cox, declare as follows:

1. I am the sole inventor of the above-referenced patent application and am familiar with the application. I am a skilled artisan in the fields of molecular and cellular biology and have been involved with the experiments described in paragraphs 4-6 below.

2. This Declaration is being submitted in conjunction with an Amendment and Response to an Office Action having a mailing date of November 28, 2006.

3. The following discussion is provided to supplement Applicants' response to the Examiner's rejection of Claims 25 and 44-46 under 35 U.S.C. § 103. Specifically, the data presented in the following paragraphs demonstrate that the present inventor and colleagues have constructed a cysteine mutein of erythropoietin (EPO) that falls within the scope of the present claims, including within the scope of the elected species, and have shown that the mutein is biologically active in an *in vitro* cell-based proliferation assay for EPO activity. Moreover, the following data demonstrate that modification of the free cysteine residue in the mutein with polyethylene glycol (PEG) does not significantly inhibit the biological activity of the mutein.

4. Using the methods and techniques described in Examples 1 and 2 of the above-referenced application, a mutein containing a single cysteine substitution was constructed in the human EPO gene and was expressed using an insect cell expression system. The reference to position numbers is made with regard to SEQ ID NO:2 of the specification, which represents the

amino acid sequence of the mature EPO protein (see Example 2). The cysteine mutein constructed was an insertion following the last amino acid of the mature protein (referred to as *167C).

5. The mutein described in paragraph 4 above was expressed as a secreted protein in insect cells using the EPO signal sequence and tested for biological activity *vs.* a wild-type EPO control protein (obtained from R&D Systems, Inc., Minneapolis, MN) in an *in vitro* cell-line based proliferation assay. The *167C EPO cysteine mutein described in paragraph 4 was purified to homogeneity. The *167C mutein was modified with polyethylene glycol ("PEGylated") using techniques substantially similar to those described in Example 1 of the above-identified application. The PEGylated form of the *167C cysteine mutein was purified away from any unmodified material. The purified cysteine mutein and the purified PEGylated cysteine mutein were assayed for biological activity *vs.* a wild type EPO in an *in vitro* cell-line proliferation assay using the human UT7/epo cell line. The purified cysteine mutein and the purified PEGylated cysteine mutein were biologically active. The EC_{50}s of the purified cysteine mutein and the purified PEGylated cysteine mutein were within 2-fold of the EC_{50} of the wild-type EPO control.

6. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Date: February 28, 2007 By: _____

George Cox